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The Importance of Rivers in Protected Areas: Macroinvertebrate Sampling Reveals the Impact of Humans and Highways on Water Quality

Marie-Claire Harrison
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The Importance of Rivers in Protected Areas: **Macroinvertebrate Sampling Reveals the Impact of Humans and Highways on Water Quality**

Author: Harrison, Marie-Claire
Academic Director: Silva, Xavier Ph.D
Project Advisor: Alexandra Hoeneisen
Pomona College
Biology
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Abstract

The water quality and level of contamination of two rivers in a cloud forest ecosystem in the Mejía region of Ecuador, the Tupí River and the Pilatón River were assessed through benthic macroinvertebrate sampling at various sites along the rivers. Many different biological indices were then used to assess water quality at each site. Pollution in the form of grey water, black water, and petroleum negatively impacted the water quality of the both rivers as they pass through the town of La Esperie. Differences in river structure and size also played a role in determining the prevalence of certain benthic macroinvertebrates: the bigger size and better oxygenation of the Pilatón allowed for more abundance of macroinvertebrates, and a higher percentage of certain pollution sensitive taxa. Therefore, differences between testing sites were not solely due to contamination. Overall, the water quality of the rivers was good to excellent before the town of La Esperie, and moderately to slightly contaminated afterwards, suggesting that waste management remains an issue in this region.

Resumen

El autor examina la calidad y nivel de contaminación del agua a través de muestra de macroinvertebrados en dos ríos en un bosque nublado en la región Mejía de Ecuador: el Río Pilatón y el Río Tupí. Contaminación por aguas negras y grises, y derivados de petrolero tienen un impacto negativo en la calidad de los dos ríos cuando pasan por el pueblo de La Esperie. Diferencias en la estructura y el tamaño de los ríos juegan un papel en la composición de las comunidades de los macroinvertebrados: el tamaño más grande del Río Pilatón permite que existe más macroinvertebrados y un porcentaje más grande de algunos familias que son sensible a la contaminación. Entonces, la contaminación no es la única que afecta a estos organismos. En general, la calidad de agua fue entre excelente y buena antes del pueble de la Esperie, y contaminada ligeramente a moderadamente después del pueblo. Eso indica que el gestión de residuos todavía es un problema en este región.

ISP Topic Codes: 601, 615, 627

Keywords: Water quality, bioindication, macroinvertebrates

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Introduction

La Hesperia Natural Reserve

La Hesperia Natural Reserve covers 798 hectares of land in the Mejía region of Ecuador. This land is used for organic agriculture and as grazing land for horses and cows, but most of it is left untouched in order to protect the integrity of this unique coastal cloud forest habitat and the ecosystems that exist there. Therefore, primary and secondary forest are primarily what compose La Hesperia and the surrounding area (Biosfera CIA LDTA., 2008). This land contains two rivers: the Tupí, and the Pilatón (Appendix H). The Tupí is a small stream that runs down the hill and is surrounded by forest and some agriculture until it reaches the town of La Esperie. When it reaches the road there, it pools into a man made pond. After this artificial dam, it runs under the road and into the Pilatón River, which runs parallel to the road.

Since it runs next to the highway and through a couple towns such as Tandapí, La Esperie and Santo Domingo, the Pilatón is exposed to a much different environment than the Tupí. A test of water quality from 2008 found traces of fecal matter, pathogens, electric conductivity, and aluminum in this river (Biosfera CIA LDTA., 2008). In particular, the amount of fecal coliforms was unquantifiable since it was so high, and can be attributed to the disposal of grey and black water into the river from the communities of Tandapi and La Esperie (Biosfera CIA LDTA., 2008). The Tupí is not exposed to the same sort of urban pollution as the Pilatón, however it still may be exposed to fertilizer run off from agriculture nearby, and is doubtlessly affected by the dam and artificial pond in La Esperie.

They are also different rivers in shape and structure: the Pilatón is much larger and wider and has a much faster flow of water than the Tupí, which is a narrower mountain stream with a much lower volume of running water. The same 2008 study found that levels of oxygen were high in the Pilatón at 8 mg/L due to its course over rocky streambeds (Biosfera CIA LDTA., 2008). Although there have no official studies on the oxygen content of the Tupí, it can be assumed due to its lower volume of water, slower flow, and course over a variety of substrates instead of just over rocks would contribute to lower oxygen levels. Finally, the Tupí has much more cover and decomposing organic matter from surrounding flora than the Pilatón. Due to these differences, the two rivers harbor different communities of macroinvertebrates that exist regardless of differences in water quality.

La Esperie, or the larger area of Parroquia Manuel C. Arroga is an area with 3,132 inhabitants (Biosfera CÍA. LTDA., 2008). Of these inhabitants, 82.4% live in poverty as measured by NBI. Consequently, only 60.4% have indoor plumbing, and only 29% have regular garbage collection services (Biosfera CÍA. LTDA., 2008). Since rivers are an easy place to dispose of waste, this may also affect the Pilatón. Additionally, the Toachi-Pilatón hydroelectric project, which involves a series of dams and rerouting of rivers in order to generate electric power (Biosfera CÍA. LTDA., 2008). The construction of the dams begin after La Esperie for the Pilatón, and do not involve rerouting the river, and therefore may not affect the river at the testing locations in La Esperie as much as at other locations.

The Importance of Cloud Forest Ecosystems in Ecuador

Cloud forests such as the one that the La Hesperia Natural Reserve contains play a vital role in the health of many surrounding ecosystems. This is because they are excellent sources of water for the surrounding watershed; by intercepting cloud and rain, they increase precipitation and capture this water through various mountain streams (Hamilton, 1995). The Tupí as a mountain stream that runs down towards the coast is an example of this mechanism. Additionally, they contain many endemic species due to their unique habitat (Hamilton). The protection of these unique and vital regions is essential, and since they are a source of water, their pollution and degradation can greatly impact the surrounding area.

History of Water Quality Analysis via Macroinvertebrates

Water of high quality is a key part of a well functioning ecosystem. Pollution of water systems can take many forms: chemical waste from factories, petroleum by-products, fertilizer run off, and waste water from towns can all impact water quality and the organisms living in and around rivers (Peréz, 2003). As rivers have become increasingly contaminated from these and other reasons, demand for a protocol for water quality testing that is quick, precise and accurate has increased.

At first, chemical methods that focused on microorganisms were more prevalent to determine water quality, as they could quickly pinpoint the sources of pollution and were relatively quick and straightforward. However, in 1908, Kolkwitz and Marrson developed a system based on varying levels of decomposition and waste in different zones and which examined biological communities in order to establish water quality (Peréz, 2003). Later, this was expanded to a more widespread focus on diversity, with measures such as species richness, uniformity, and abundance in order to assess water quality. Then, in 1955, Beck proposed that a biotic index in which species were ranked by their tolerance to pollution would be useful for assessing contamination (Beck, 1955). This has since been realized in many different biotic indexes for different regions throughout the second half of the 20th century.

Macroinvertebrates are an ideal community for a biotic index since they are relatively sedentary, spend some or all of their life cycle in the water, and are easy enough to identify without a microscope (Lenat, 1988). They are therefore good indicators of localized contamination in water sources and can be expanded to infer water quality. Although many different indices have been developed, the Biological Monitoring Working Party (BWMP) is one of the most well known and commonly used in the United States (Peréz, 2003). This has been adapted for use in Columbia by Peréz (2003) under the name BMWP/Col. Other methods commonly used include Average Score Per Taxon (ASPT), Pertcentage of Emphemoptera, Plecoptera and Trichoptera (%EPT), Sensibilidad (Carrera et. al 2001), and the Family Biotic Index (Hilsenhoff, 1988). However, the BMWP index, and by extension the BMWP/Col. has been shown to work very well for assessing water quality of rivers and streams (Lenat, 1988).

What factors determine communities of aquatic macroinvertebrates?

Ecological communities of macroinvertebrates in rivers can be impacted by a variety of factors, and therefore assessing water quality through macroinvertebrate populations can be a complex process in which many factors must be taken into consideration. The turbidity, dissolved oxygen, temperature, air pressure, salinity, amount of organic matter, amount of CO_2, the pH, and various other nutrients can all impact macroinvertebrate communities (Peréz, 2003). Therefore, macroinvertebrate communities can change due to factors that are separate pollution and habitat disturbance.

Many different authors have explored the interplay of these factors and habitat disturbance. For example, fertilizer run off can impact macroinvertebrate communities by diminishing the amount of oxygen available for these organisms (Hart et al., 2004). This impacts aquatic fauna because differences in oxygen levels can diminish the numbers of macroinvertebrates that require high levels of oxygen, such as mayflies (Connolly et al., 1983). Additionally, differences in pH and levels of certain metals such as copper and zinc greatly impact the ability of macroinvertebrates to thrive in rivers; acidification and toxic metals can cause a decrease in abundance and diversity of aquatic fauna (Hirst et al., 2002). Scientists must consider these factors when measuring macroinvertebrate communities to distinguish between the effects of different environments and the effects of pollution and habitat disturbance.

The Impact of Precipitation

One factor that is particularly significant in cloud forest ecosystems is the effect of rain on macroinvertebrate sampling. Peréz (2003) suggests not sampling after heavy rain as this can wash away macroinvertebrates and make their abundance and diversity lower than it is in reality. But does this concept still apply in the cloud forest, where rain is more frequent? According to a study done by Vega et al. (2014), big storms decrease the abundance and diversity of macroinvertebrates by disturbing their habitat. Furthermore, Jacobsen & Encalada (1998) elaborate that more macroinvertebrates and more taxa richness were found in Ecuadorian highland streams in the dry season than in the wet season. However, in another article, Jacobsen elaborates that the wet season increases the number of macroinvertebrates and species richness in polluted downstream sites, but decreases the abundance and species richness at upstream, less polluted sites (Jacobsen, 1998). Therefore, differences in macroinvertebrate populations corresponding to water quality are more pronounced during the dry season, making it the better time to test the water quality of rivers and the effects of pollution on these rivers.

Objective and Hypothesis

The objective of this study was to analyze the impact of the town of La Esperie and the highway on the quality of the water in the Tupí and Pilatón rivers in comparison to the benefit that the La Hesperia Reserve conferred on the upper parts of the Tupí River for the macroinvertebrates living there. I hypothesized that the town and the highway would have a negative impact on the abundance and species richness of macroinvertebrates in these rivers, as well as reduce the quality of the water as demonstrated by various indices. Additionally, this study could provide insight into the impact of the Toachi-Pilatón

Hydroelectric Project on the water quality of the Pilatón and the progress of waste management in La Esperie and Tandapi.

Methods and Materials

The protocol followed to collect macroinvertebrates was an adaptation of the Single Habitat Sample Approach from the EPA's "Rapid Bioassesssment Protocols For Use in Streams and Wadeable Rivers" (Barbour et al., 1999). Suggestions were also taken from Peréz (2003) in his article *Bioindicación de la calidad de los aguas en Columbia*. Air and water temperature, bank, canopy coverage (rated from very covered, partially covered and no cover), substrates, water transparency, and velocity of the river (slow, moderate, or fast) were recorded before collecting macroinvertebrates.

To collect macroinvertebrates for analysis, the floor of the river and substrates were disturbed for 60 seconds with a dip net or sieve 20cm downstream. Nearby rocks and dead leaves were scrubbed into the net during this process. The net was placed primarily in riffles and runs but also in calmer, sandy pools and on the banks on each side of the river. This process was repeated in 7 different locations (labeled 1-7), which varied in terms of substrate, coverage, and velocity of the stream to ensure the highest diversity of macroinvertebrates was captured. The area sampled was a 10-15m stretch along each site. Fiver sites were tested: the Upper Tupí (A), lower Tupí (B), Upper Pilatón (C) and the intersection of the Tupí and the Pilatón (D), and downstream on the Pilatón (E) (Appendix H). To ensure the same amount of effort was put into each site, 1.5 hours were spent collecting the macroinvertebrates at each location (A, B, C D and E), and each location was tested at least 4 times following these parameters. Some of the earlier collections could not be quantitatively compared to later ones due to a period of heavier rain from November 8th to November 13th, as well as inexperience yielding lower results of macroinvertebrates. Macroinvertebrates were usually collected from 9:30-11:00am in the morning, and sometimes also from 6:00-7:30am.

The macroinvertebrates found at each sample site were then separated from substrates and organic material with forceps and put into an individual jar filled with water. They were then transported back to the La Hesperia lodge to be identified in the afternoon. Once back at the lodge, they were identified to the family level using the book *Bioindicación de la calidad de agua en Colombia* by Gabriel Alfonso Peréz (2003).

Many different indices were used to analyze the data at each sampling site, with each 1.5-hour set of 7 samples considered to be a complete sample of the macroinvertebrate populations. The samples taken at the Tupí and the Pilatón on November 8th and November 9th were combined to make one data set since each was only tested for 45 min on these days.

- *Biological Monitoring Working Party in Columbia,* or BMWP/Col: This system took common families in the Columbia area, determined their sensitivity to pollution, and gave them a score from 1-10, with 10 being most sensitive, and summed them. This number determined water quality: the higher, the better quality. Developed by Peréz (2003).

- *ASPT* (Average score per taxon) used the above method to assign scores to each family of macroinvertebrate, and then divided that by the number of taxa (in this case, family).

- *Percent EPT* (the percent of Ephemoptera, Plecoptera, and Trichoptera) has been commonly used as a way to evaluate water quality, with higher percentages meaning higher water quality since these groups tend to be more sensitive to pollution (Lenat, 1988).

- *Taxa richness*, as a simple measure of diversity, is a reliable measure of water quality (Lenat. 1988).

- *The Family biotic index* (FBI) was developed by Hilsenhoff (1988) was another method that assigned scores to families of macroinvertebrates in order to assess water quality; however this one took into account the abundance of each family. This method differs from the others in that a higher score meant lower water quality, while for the other indices a higher score meant higher water quality.

- S*ensibilidad*, which was developed as a way to assess water quality on the coast of Ecuador, was also used to assess the quality of the water, however since it does not include many of the groups that were observed, this method was more imprecise than the others (Carrera et al., 2001).

- *Shannon diversity* (H), a statistical measure of diversity was used to assess to total diversity of each site by combining all samples and using the formula:

  $$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

  Where P_i is the number of individuals in each family. (Hughes, 1978).

- *Simpson's diversity index* (1-D) was also used to evaluate the total diversity macroinvertebrate populations at each testing site using the formula.

  $$1 - D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$$

  Where n is number of organisms per family, and N is total number of organisms. (DeJong, 1975).

- Sample coverage of the entire dataset of each site was calculated using Chao and Jost's data completeness calculator (Chao & Jost, 2012).

The average score per sample for each site was found for all indexes except for the Simpson's and Shannon's Diversity indexes. For these averages, data from the date 11/10 was left out for the Tupí (site A), and from the dates 11/12, 11/15, 11/8 and 11/9 for the Pilatón (site C), due to inexperience sampling and heavy rains leading to lower amounts and diversity of macroinvertebrates being collected. For the Family Biotic Index, the family Leptohyphidae was assumed to have the same score as Heptageniidae due to similar sensitivity, and Hyallidae was assumed to be the same as Gammaridae since they are both Amphipods. But in general, if a family was not included in the index, it was excluded from the calculation.

Results

Initial observations of the substrate and surroundings of each site, as displayed in Appendix G, provided several significant insights. Sites A and B had the most cover, organic material and dead leaves from the surrounding forest, while Site C had the most clean substrates (hardly any algae or aquatic vegetation), Site D had the most human-generated waste (organic and inorganic), and Site E had the most algae and aquatic vegetation. The Pilatón also has a much faster flow of water than the Tupí, and is much wider: the former is around .5-1.5m while the latter is around 10m.

The water quality assessment based on the BMWP/Col. index indicated that water was best at the lower Tupí (B), but still good at the upper portion of the Tupí (A) and at the upstream site of the Pilatón (C) (Table 1, Appendix A). However, it is doubtful at the intersection of the Pilatón and the Tupí (D) and only acceptable at downstream site of the Pilatón (E). This suggests that site D is moderately contaminated and site E is slightly contaminated (Peréz, 2003).

Table 1: Assessment of water quality using the BMWP/Col. Index developed by Peréz (2003) at 5 different sites along the Pilatón and Tupí, Mejía, Ecuador, 2016.

	BMWP/Col	Quality	Standard Deviation
A: Upper Tupí	110.25	Good	15.15
B: Lower Tupí	119.5	Good	15.67
C: Upstream Pilatón	103	Good	6.55
D: Intersection of Pilatón and Tupí	37.0	Doubtful	17.57
E: Downstream Pilatón	74.75	Acceptable	7.23

Average Score Per Taxon (APST) scores from this method can be compared similarly: at 7.75 and 7.52 respectively, sites B and A have the highest water quality. C falls slightly lower at 7.2, E still lower at 6.475 and finally D, by far the lowest at 5.09 (Figure 1, Appendix A). This suggests the same pattern of water quality as the BMWP/Col. scores.

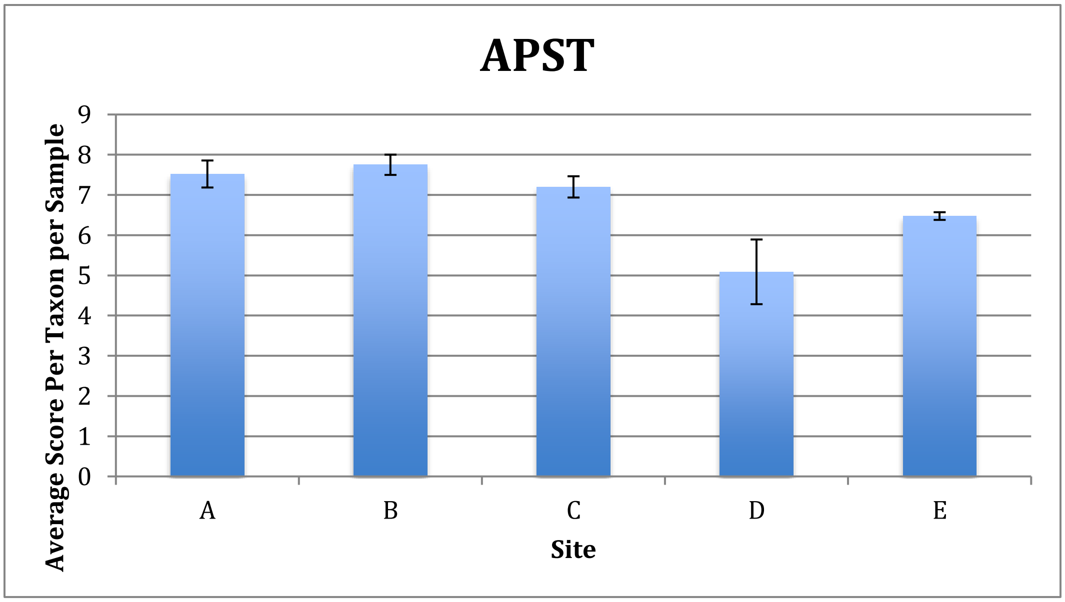

Figure 1: The average of the Average Score per Taxa (APST) per sample using the BMWP/Col. index (Peréz, 2003).

Taxa richness followed a similar pattern, with sites A and B having the most different families of macroinvertebrates, C having slightly less, E even less, and D having the lowest number of families of macroinvertebrates (Figure 2). The family Biotic Index implied the similar results according to the water quality key for this index, where a higher score indicates lower water quality, except that site B had lower water quality than A and C according to this index (Figure 3, Appendix B).

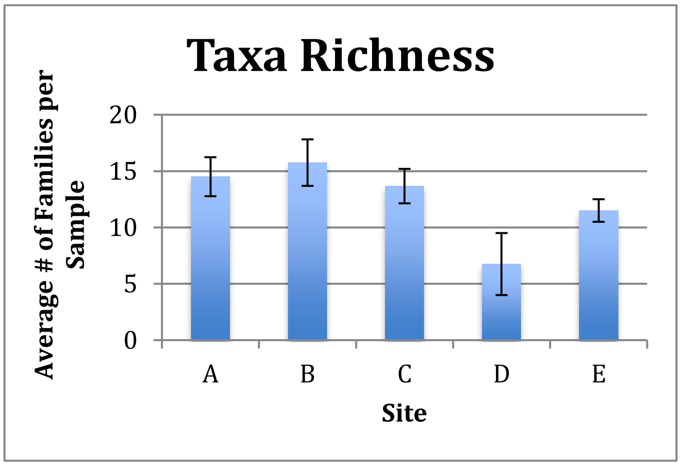

Figure 2: Average taxa richness per sample (number of families) at each site. Error bars display standard deviation.

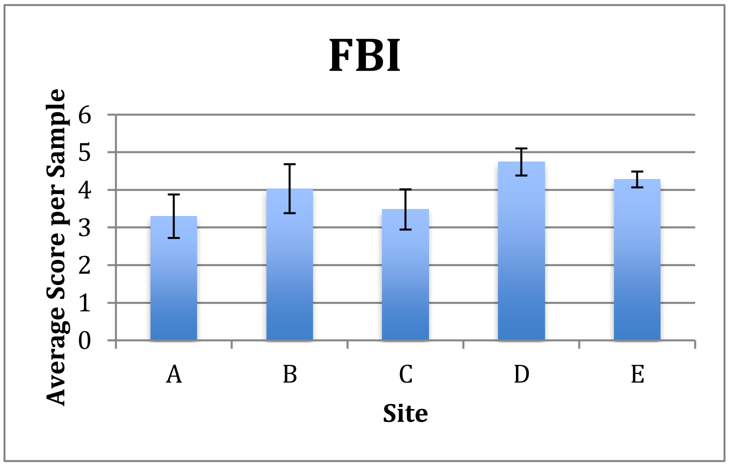

Figure 3: Average Score per sample according to the Family Biotic Index at each site. Error bars display standard deviation.

Percentage of macroinvertebrates in the Ephemoptera, Plecoptera and Trichoptera families (%EPT) was highest at sites C and E, with sites A and B having similar, lower percentages, and D having the lowest amount of EPT organisms (Figure 3). This does not follow the aforementioned pattern. Similarly, macroinvertebrates were much more abundant at the Pilatón sites (C and E) than at the Tupí sites (A and B), with the intersection (D) remaining the lowest (Figure 4).

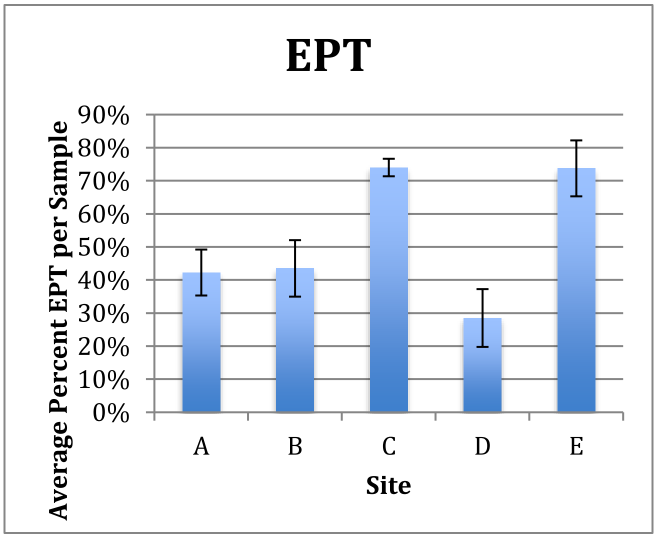

Figure 3: Average percent of Ephemoptera, Trichoptera and Plecoptera per sample at each site. Error bars display standard deviation.

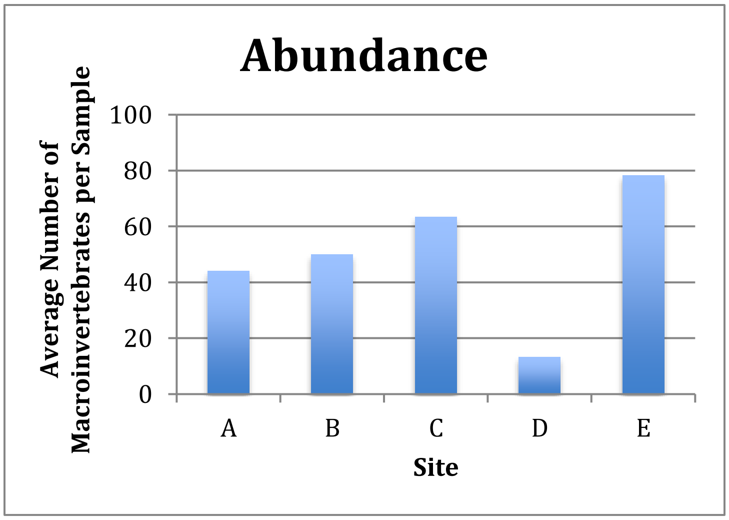

Figure 4: Average number of Macroinvertebrates collected per sample at each site.

The Sensibilidad index showed a much higher water quality at sites A, B and C as compared to E and D, which follows a similar pattern as the BMWP/Col. index (Figure 4).

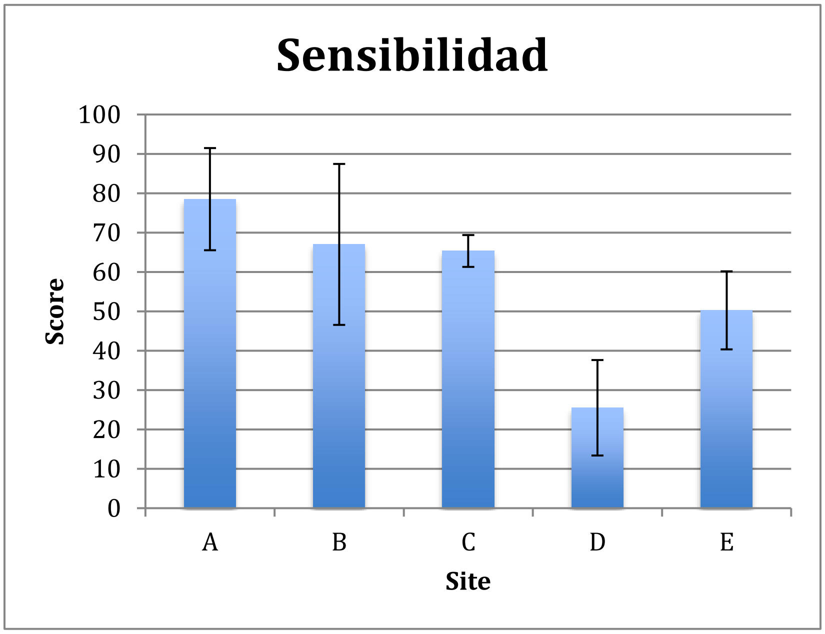

Figure 5: The average water quality score per sample at each site based on the Sensibilidad index (Carrerra, 2001). Standard deviation is displayed as error bars.

The communities of macroinvertebrates varied between each site. Sites A and B shared many dominant species such as Hydropsychidae and Ptilodactylidae, while sites C and E were clearly dominated by Leptohyphidae (Appendix D). Site D had a dramatically different composition than any of the other site, with Chironomidae dominating (Appendix D).

Using all samples recorded at each site, the Shannon Diversity index (H) and Simpson's diversity index (1-D) showed that diversity decreased in the Pilatón River (C and E), and at the intersection of the Pilatón and the Tupí (Table 2).

Table 2: Shannon's Diversity Index (H) and Simpson's Diversity (D) calculated from the total sampling data from each site.

Site	Shannon's Diversity index (H)	Simpson's Diversity index (1-D)
A	2.7	0.91
B	2.8	0.91
C	2.25	0.85
D	2.23	0.86
E	2.06	0.83

Sample coverage was also estimated, using Chao, A., Ma, K. H., and Hsieh, T. C. (2016) iNEXT (iNterpolation and EXTrapolation) Online, to be between 85%-96% for all samples combined at each site, with site D having the lowest coverage of 85% (Table 3). Further extrapolation and interpolation data can be found in Appendix E.

Table 3: Completeness of the dataset calculated from the total sampling data from each site.

Site	Estimator of Sample Coverage
A	0.9642
B	0.9689
C	0.9511
D	0.8447
E	0.984

When comparing the average scores of all indices for each river, the Tupí has, on average, higher water quality across all measures except %EPT. %EPT was significantly higher on average at the Pilatón sites than at the Tupí sites.

Table 4: Comparison of the average scores of the Tupí (Sites A and B) with the average scores of the Pilatón (Sites C and E) according to various indices.

River	APST	BMWP/Col	Taxa	Sensibilidad	FBI	%EPT
Tupí	7.6354125	114.875	15.125	72.75	3.66625	0.42875
Pilatón	6.8375	88.875	12.58333333	57.79166667	3.87875	0.73875

Abundance of macroinvertebrates significantly decreased during a period of heavy rain throughout November 7th to November 13th (Figure 6).

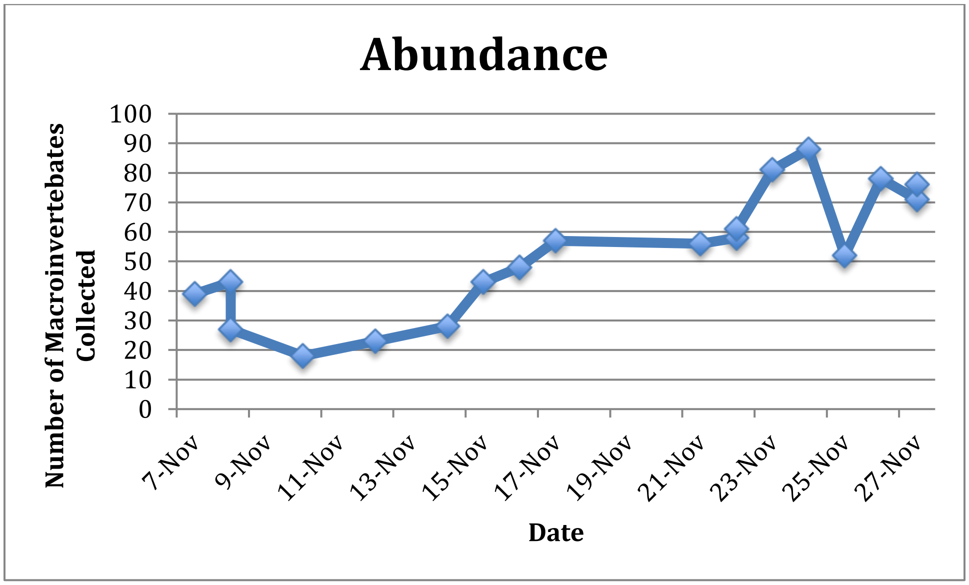

Figure 6: Average abundance at each site as varied by collection day. Site D was excluded due to much lower numbers than any other site.

Taxa richness per sample at site C was also significantly lower during this period of heavy rain (Figure 7).

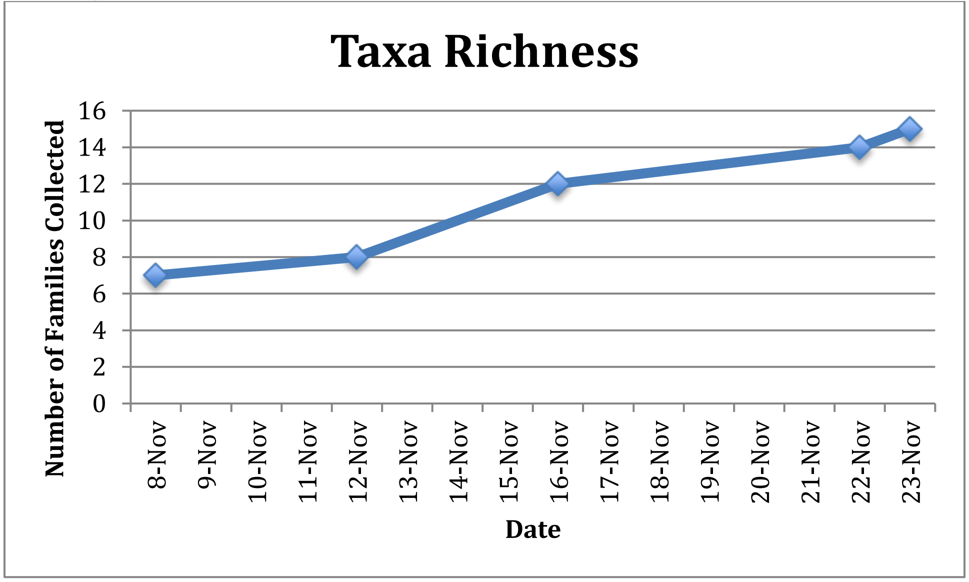

Figure 7: Taxa richness as varied per day at site C (Upper Pilatón), the 15th was excluded due to inadequate sampling time.

Discussion

Effect of the Town of La Esperie

The effect of the town of La Esperie on both the Tupí and the Pilatón River is abundantly clear based on many of the biological indices. Although the rivers started out with high water quality at their upstream sites according to (A, B, and C) both the Tupí and the Pilatón were substantially worse at the downstream sites (D and E) than at the upstream sites according to the BMWP/Col. index score and ASPT (Table 1, Figure 1). Although the standard deviation was fairly high for this index, the differences were still significant and extended past the standard deviation (Table 1). Percent EPT, the Family biotic index, and taxa richness also showed significant differences between upstream and downstream water quality, with downstream water quality being worse (Figures 1-3). However, the Pilatón has less of a difference in water quality between downstream and upstream sites (C and E) than the Tupí does, suggesting that the town impacts it less than the Tupí (Table 1, Figures 1-4). This is most likely due to the larger size and therefore greater ability to withstand pollution of the Pilatón (Peréz, 2003).

The lower water quality can be explained by the fact that the town generates a lot of waste in the form of discarded organic and inorganic materials, and grey and black water. The fact that only 60.4% of the residents of La Esperie and surrounding areas have indoor plumbing, and only 29% have regular garbage collection services means that the river is often used as a place to discard waste and waste water (Biosfera CÍA. LTDA., 2008). The organic and inorganic waste such as orange peels, chicken bones, plastic bags, gasoline, and pieces of paper that was observed reinforces this hypothesis (Appendix F). Fungicides and herbicides used on local crops can also have a negative impact on macroinvertebrate populations and may contribute to the lower water quality; however this impact would not be specific to the town of La Esperie (Cuppen et. al, 2000).

In particular, the Family Biotic Index can be used to assess the negative impacts of organic waste on macroinvertebrate populations (Hilsenhoff, 1988). This explains how waste and runoff from pastureland, as well as grey and black water, although not toxic in the same way as metals such as aluminum, can impact water quality. Since fecal coliforms were found to be at extremely high levels in the Pilatón in a 2008 study, it is likely that these pathogens contribute to the poor water quality of the Pilatón as it passes through the town of La Esperie (Biosfera CIA LDTA., 2008). Another study found that organic pollution, although it can provide some food sources for macroinvertebrates, is "generally damaging" to their populations, especially when it at levels that it lowers the amount of dissolved oxygen in the water (Simon & Buikema, 1997). This is the most likely and most abundant source of pollution for the Pilatón River. However, the Tupí does not appear to be affected by organic pollution until it reaches the highway, since water quality at Site A and Site B is similar according to several indices: BMWP/Col., %EPT and Taxa Richness (Table 1, Figure 1, Figure 3). This suggests that pastureland and farming runoff are minimal in the La Hesperia Reserve.

Grey and black water from the town La Esperie may also contain many other pollutants that impact the Pilatón and the intersection of the Tupí with the Pilatón. Chlorine, as found in many laundry detergents and bleaches, is especially damaging to water quality since once it dissolves in water, it becomes volatile and can chlorinate organic compounds which persist much longer than free chlorine and can be more toxic to aquatic life (Brungs, 1973). The amount of chlorine in the water is referred to as the Total Residual Chlorine (TRC), and is acutely toxic to all organisms at high concentrations. Some macroinvertebrates are more sensitive than others, such as mayfly nymphs, which have been reported to exhibit acute toxicity at concentrations of 5 µg/l in South Africa, which is much lower than the effluent standard of 100 µg/l (Williams et al, 2003). Therefore, this may be a source of contamination from the town of La Esperie as well, especially at site D, where less sensitive taxa were observed.

Gasoline residue was observed around the edges of the Pilatón, especially at site D, and so this is as well expected to impact the water quality of the Pilatón as it passes through La Esperie (Appendix F). Petroleum-derived hydrocarbons are considered to be "one of the main pollutants of aquatic ecosystems", but are less visible than large petroleum spills, and can induce abnormalities and toxicity in many macroinvertebrates (Rodrigues et al, 2010). Due to the close proximity of the Pilatón to the highway, diesel, petroleum and gasoline runoff certainly contribute to the water quality of the river, and likely is the main reason that it tended to have a lower water quality score according to all indices tested except percent EPT (Table 4). It also had less diversity of macroinvertebrates according to both Shannon's and Simpson's Diversity indices (Table 2). This implies that the water quality of the Pilatón is less than that of the Tupí.

Inherent Differences Between the Tupi and the Pilatón

The Tupí and the Pilatón are inherently different rivers, and this is reflected especially by the population composition and abundance of macroinvertebrates in each river. Due to its large volume and fast flow of water, the oxygen content can be assumed to be much higher than the slower moving Tupí (Appendix G). Indeed, when tested in 2008, the river was observed to have 8 mg/l of dissolved oxygen, which is quite high (Biosfera CIA LDTA., 2008). However, this is due to its structure, and not to low level of pollutants (Biosfera CIA LDTA., 2008). Therefore, macroinvertebrates at these sites were much more abundant, and the frequency of EPT taxa was much higher at sites C and E than at the Tupí sites of A and B (Figure 3, Table 4). EPT, although excellent for judging changes in water quality over time at the same site, can widely vary based on eco-region, and therefore is less reliable for comparing between rivers (Lenat, 1988).

The impact of a faster flow and therefore higher oxygen content is also supported by the fact that the Tupí has a slightly higher taxa richness, abundance, %EPT, and BWMP score at site B, where it is wider and has a faster flow of water than at site A (Figure 1, 3, 4, Appendix G). These changes demonstrate how water quality can be impacted by environmental factors that are unrelated to contamination. Additionally, the large width and volume of the Pilatón as well as the many uncontaminated mountain streams feeding it could allow a faster recovery time compared to the small mountain stream structure of the Tupí.

The composition of the macroinvertebrate populations at each site was indicative of the structure of each. The most prevalent macroinvertebrate at site A was Ptilodactylidae larvae, of the order Coleoptera (Appendix D). These macroinvertebrates feed on rotten wood and require 3 years for full growth, and so it makes sense that they are abundant in areas surrounded by trees with plenty of available leaves and branches falling into the water (LeSage & Harper, 1976). Elmidae and Perlidae were also dominant, which both prefer riffles habitats: this shows the variety of habitats available in this stream (Peréz, 2003). At site B, Hydropsychidae was by far the most abundant family (Appendix D). As primarily predatory macroinvertebrates that spin webs to catch smaller macroinvertebrates and plant material to consume that prefer mountain streams, it is logical that they would be more prevalent downstream, with a faster flow of water to provide more prey and plant material to capture (Wallace, 1975).

At Site C and E, Leptohyphidae and Baetide were clearly dominant species(Appendix D). This is due to their lifestyle as collector-gatherers and their dependence on high levels of oxygenation, as well as their preference for cobble-riffle habitats, where they exist in high numbers (Ramirez et al., 1998). In general, cobble riffle habitats have higher numbers of macroinvertebrates (Ramirez et al., 1998). This explains why the Pilatón had higher numbers of macroinvertebrates per sample, since it is composed of many cobble-riffle habitats, whereas the Tupí has more pools and leaf packs. The variety of structures in the Tupí allows for a greater variety of macroinvertebrates, but the fact remains that the Pilatón, as a larger, more oxygenated river, has a structure that many EPT taxa prefer.

At site D, Chironomidae was the most prevalent family of macroinvertebrate (Appendix D). Since the structure of this area is similar to that of the lower Tupí, this shows the high level of pollution of that site, since Chironomidae have a high tolerance of pollution (Peréz, 2003). Additionally, the macroinvertebrates caught in the dip net at this site were often covered in algae and slow moving, further attesting to the toxic levels of organic and inorganic pollution at this sight (Appendix G).

Impact of precipitation

Precipitation certainly did impact the abundance and diversity of macroinvertebrates collected. The amount of macroinvertebrates collected per 1.5-hour sampling period were significantly lower during the period of heavy rain between November 7-13 and increased after that time (Figure 6). Less macroinvertebrates collected meant less diversity could be observed, as demonstrated by site C (Figure 7,

Appendix D). This confirms the findings of Jacobsen & Encalada (1998), who hypothesized that during the wet season, less macroinvertebrates were collected due to the increase in river volume washing them away from their normal spots.

Effect of Hydroelectric Dam in construction

The hydroelectric dam that is being constructed after the town of La Esperie may contribute to the observed decline of water quality at site E compared to site C observed with the BMWP/Col., APST, Taxa richness, FBI, and Sensibilidad indices (Table 1, Figures 1-5). Hydroelectric dams can drastically impact the macroinvertebrate communities by decreasing abundance and driving many more sensitive genera to extinction (Jalon et al., 1994). However, this not observed at site E; many sensitive genera in the form of %EPT were as or more abundant than before the dam. In contrast, the small dam that the Tupí encounters as it reaches the road may impact macroinvertebrates more severely by raising the temperature of the surface water and disturbing the normal structure of the river (Lessard & Hayes, 2003). Indeed, the number of sensitive taxa (%EPT) greatly decreased at site D, and it is likely that the man made pond that the small mountain stream encounters before running under the road contributes to this (Figure 3). So although in the future, the dams in the Pilatón may impact water quality at La Esperie, it is impossible to discern this without macroinvertebrate tests from after the construction. It is more likely that pollution from La Esperie, as observed at site D, is more what contributed to the decline in water quality at site E.

Discrepancies between Indices: Which is best?

Several different indices were used to evaluate water quality, however it can be difficult to distinguish the significance of each finding. Overall, the BMWP/Col. was determined to be the most accurate for the Mejía Cloud Forest area, since this index was developed for the entire country of Columbia and therefore should extend to the rest of South America quite well, despite some differences in climate and environment (Peréz, 2003). In theory, the Sensibilidad index, since it was developed for the coast of Ecuador, would be the most accurate (Carrera, 2001). However, many macroinvertebrates found were not included in the index, so this method would be less accurate for determine water quality.

The other methods, such as taxa richness, ASPT, FBI and %EPT, have been in use for decades and are well established as an accurate way to measure water quality (Lenat, 1988). However, since they were developed in North America and Europe, they may not be as precise as BMWP/Col. for Ecuador. In particular, the Family Biotic Index specified a particular form of collecting macroinvertebrates that was not followed, since the protocol of BMWP/Col. was followed and the FBI called for very high numbers of macroinvertebrates collected per sample, and the exclusion of certain taxa (Hilsenhoff, 1988). Therefore, this index may be particularly inaccurate. Future studies could use chemical testing to assess the accuracy of each index.

Although Shannon’s and Simpson’s diversity indices were useful for assessing diversity of macroinvertebrate populations, diversity can be impacted by factors other than contamination (Hughes, 1978; DeJong, 1975). Furthermore, both these indices are dependent on sample size, and sample sizes varied based on river structure and contamination.

Sources of Errors

Inexperience with macroinvertebrates and therefore difficulty identifying them and collecting them efficiently was initially the biggest source of error in my results. Many less macroinvertebrates and less taxa richness was observed during the first week of collection (11/7-11/13) due to this (Appendix D). Combined with the impact of precipitation during the first week of collection, this makes the data taken then less reliable than data taken later. However, overall, the total data taken and used for diversity indices had a high amount of completeness: around 95% for all except site D (Table 3, Appendix E). D's comparatively low level of completeness makes its comparison to other sites may be less accurate.

Additionally, not all sites were tested for the same total period of time: it varied from 4-1.5 hour collections to 6-1.5 hour collections, causing a discrepancy in the completeness of the data. Only site C was tested 6 times (the rest were tested 4 or 5 times), due to the fact that earlier testing was during periods of heavy rain and yielded far fewer macroinvertebrates than later observed, and therefore the total results do not significantly differ from site E in terms of abundance. However, this could especially impact the total diversity observed at each site, since more collection time allows for more diverse macroinvertebrates to be found. Indeed, the sites that were tested for the least amount of time (D and E) displayed the least diversity (Table 3). Therefore, diversity measures that look at average or total diversity per sample are more accurate for this study. Finally, due to its large width and volume, only one bank of the Pilatón could be tested as opposed to all parts of the Tupí. This may have impacted diversity measures.

Future Studies

As mentioned above, future studies could run chemical tests on the water to reveal sources of pollution, especially for the moderately contaminated Pilatón. Although the Pilatón had many tests ran on it in 2008, changes in infrastructure that reduce the amount of grey and black water, as well urban run off, that reaches the river may have occurred, and the ongoing construction hydroelectric dam may have impacted water quality. It would be interesting to study the water quality in Tandapi to see the effect this larger town has on water quality, as the distance between Tandapi and La Esperie may allow the Pilatón to recover. And although it is fairly obvious that the Tupí has excellent water quality, the amount of organic contamination from pastureland could also be examined. Taking samples at different locations along the Pilatón and other affected rivers where the dams are placed could also assess the impact of the Toachi-Pilatón hydroelectric project; it is hard to tell the impact of the dams without data from after the dams and from rerouted rivers.

Conclusion

The impact of the highway and the town of La Esperie on the water quality of both the Tupí and the Pilatón Rivers are clearly negative. This is most likely due to organic and inorganic waste as well as urban run off, which can contain petroleum. However, differences in river structure cause changes in the composition of benthic macroinvertebrate populations that are unrelated to pollution. Additionally, periods of heavy rain impact macroinvertebrate diversity and abundance separately from contamination. Based on macroinvertebrate sampling, the water quality of the Tupí River is high until it reaches the town of La Esperie, where it is moderately contaminated. The Pilatón River also appears has low levels of contamination according to its macroinvertebrate populations until it reaches the town of La Esperie, despite its position next to the highway and exposure to grey and black water from Tandapi. Improvements in waste management and strategies to mitigate urban runoff could improve the quality of the Pilatón and reduce the impact of La Esperie on both rivers. The impact of the small dam that the Tupí encounters on its water quality also highlights the detrimental impact of dams on water quality and emphasizes the need for further water quality testing to assure the Toachi-Pilatón hydroelectric project does not damage the rivers involved. Rivers are home to many sensitive populations that reserves such as La Hesperia can protect from pollution.

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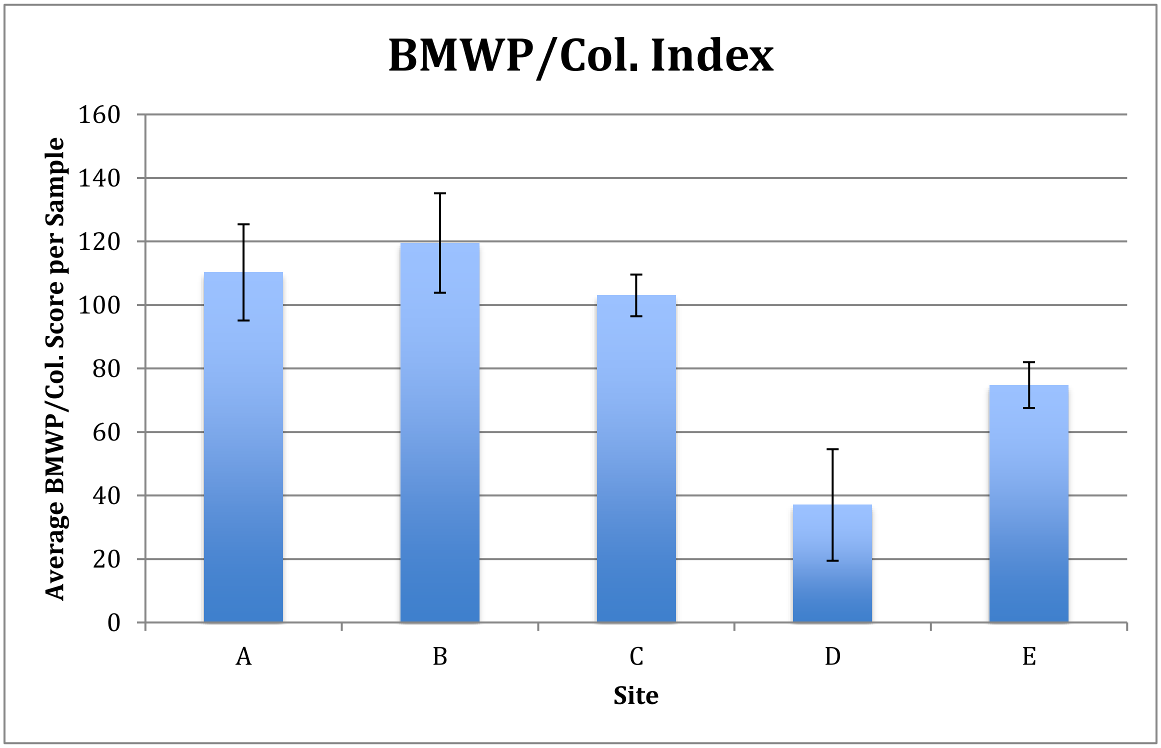
Appendix A: Biological Monitoring Working Party/Columbia, Average Score Per Taxon, Taxa richness and percent EPT.



Supplemental Figure 1: Average Biological Monitoring Working Party in Columbia scores for each site, per sample.

Supplemental Table 1: Meaning of the water quality scores from macroinvertebrate assessments in streams using the Biological Monitoring Working Party/Columbia index. Adapted from Table 4.3 in *Bioindicación de la calidad de las aguas en Columbia* by Gabriel A. R. Peréz (2003).

Quality	BMWP/Col. Score	Meaning
Good	>101	Clean to very clean water
Acceptable	61-100	Slightly contaminated water
Doubtful	36-60	Moderately contaminated water
Critical	16-35	Very contaminated water
Very Critical	<15	Severely contaminated water

Supplemental Table 2: The average of the Average Score per Taxa (APST) per sample using the BMWP/Col. index (Peréz, 2003).

	Average APST per Sample	Standard Deviation
A	7.520825	0.335933608
B	7.75	0.251661148
C	7.2	0.264575131
D	5.0875	0.804544385
E	6.475	0.095742711

Supplemental Table 3: The average of EPT percentages for each site, per sample.

	EPT	Standard deviation
A	42%	7%
B	44%	9%
C	74%	3%
D	29%	9%
E	74%	8%

Supplemental Table 4: The average number of Taxa (families in this case) per sample .

	Taxa	Standard Deviation
A	14.5	1.732050808
B	15.75	2.061552813
C	13.66666667	1.527525232
D	6.75	2.753785274
E	11.5	1

Appendix B: Family-level Biotic Index

Supplemental Table 5: Key for interpretation of the Family Biotic Index (Hilsenhoff, 1988). Adapated from "Table 2: Evalulation of water quality using the family-level biotix index" in the article "Assessment of Organic Pollution with a Family-Level Biotic Index" by William L. Hilsenhoff (1988).

Family Biotic Index Score	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very good	Possible slight organic pollution
4.26-.500	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very poor	Severe organic pollution likely

Supplemental Table 6: Assessment of water quality at each site using the Family Biotic Index (Hilsenhoff, 1988).

Site	FBI	Standard deviation	Water Quality
A	3.3	0.578330932	Excellent
B	4.0325	0.651274392	Very good
C	3.48	0.535070089	Excellent
D	4.7425	0.360404865	Good
E	4.2775	0.210455854	Good

Appendix C: Sensibilidad Index

Supplemental Table 7: The average water quality score per sample at each site based on the Sensibilidad index (Carrerra, 2001).

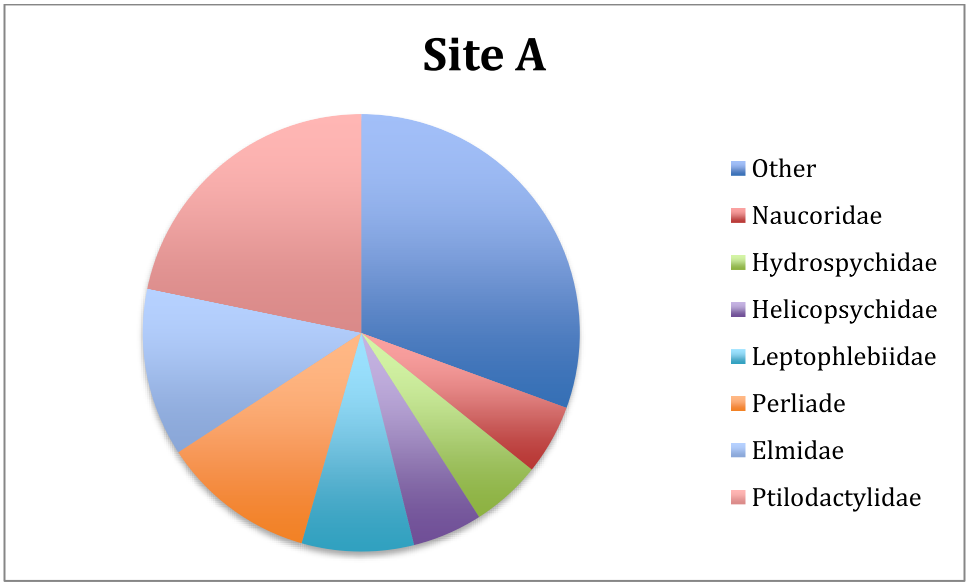
	Sensibilidad	Standard Deviation	Water Quality
A	78.5	12.97433364	Good
B	67	20.4450483	Good
C	65.33333333	4.041451884	Good
D	25.5	12.12435565	Bad
E	50.25	9.912113801	Regular

Appendix D: Composition

Supplemental Table 8: All of the macroinvertebrates found at Site A (Upper Tupí River, Mejía, Ecuador), arranged from least abundant to most abundant. 2016

A	11/7	11/10	11/14	11/17	11/25	Total
Atriplectidae	1	0	0	0	0	1
Oiligonueridae	1	0	0	0	0	1
Calamocseratidae	0	1	0	0	0	1
Ampullaridae	0	0	1	0	0	1
Coenagnoidae	0	0	0	1	0	1
Leptoceridae	0	0	0	0	1	1
Caenidae	0	0	0	0	1	1
Thiaridae	2	0	0	0	0	2
Psychodidade	1	1	0	0	0	2
Tabanidae	2	0	0	0	0	2
Grynidae	2	0	0	0	0	2
Sialidae	1	0	1	0	0	2
Tipulidae	0	0	1	1	0	2
Hydroptilidae	0	0	0	2	0	2
Corydalidae	0	0	0	0	2	2
Dytiscidae	2	0	0	0	1	3
Gomphidae	0	0	0	2	3	5
Leptohyphidae	0	2	1	1	2	6
Baetidae	0	0	2	3	1	6
Chironomidae	8	0	0	0	0	8
Gerridae	4	0	0	0	4	8
Naucoridae	1	1	2	5	1	10
Hydrospychidae	0	1	6	3	0	10
Helicopsychidae	0	0	1	4	5	10
Leptophlebiidae	1	1	3	2	9	16
Perliade	8	3	2	6	3	22
Elmidae	1	0	1	16	6	24
Ptilodactylidae	5	8	7	12	10	42

Total	40	18	28	58	49	193

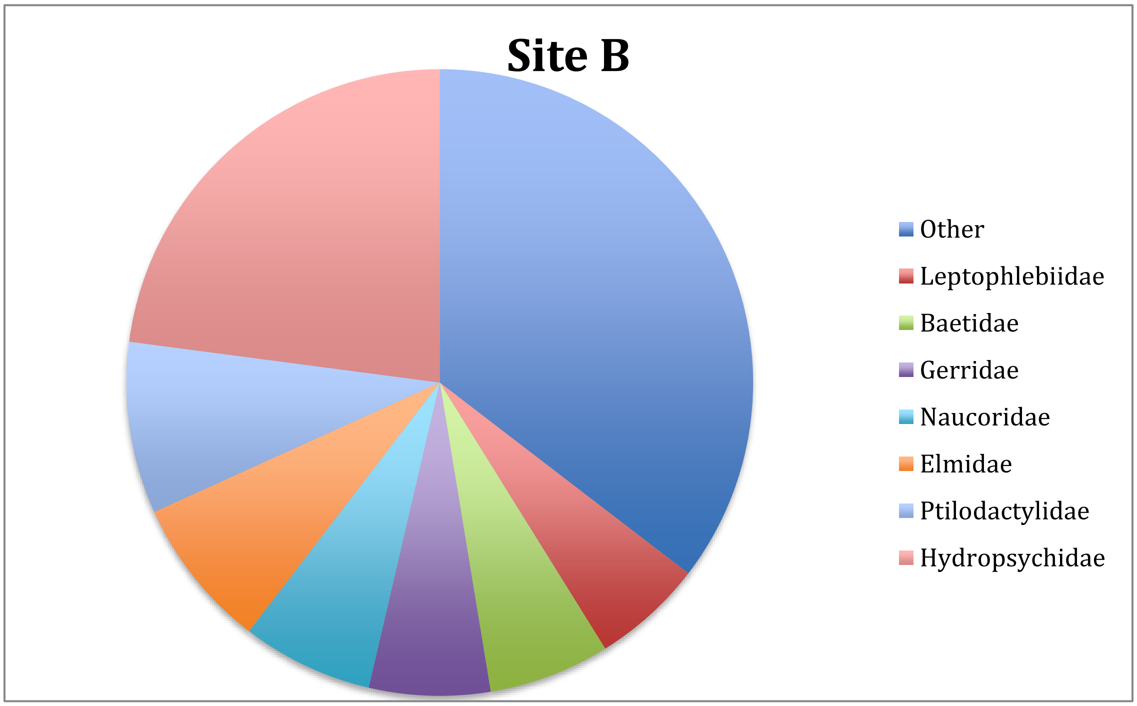


Supplemental Figure 2: The 7 most abundant species of macroinvertebrates at Site A, Upper Tupí, Mejía, Ecuador. N=193.

Supplemental Table 9: All of the macroinvertebrates found at Site B (Lower Tupí River, Mejía, Ecuador), arranged from least abundant to most abundant. 2016.

B	11/8+11/9 B	11/15 B	11/21 B	11/22 B	Total
Bselostomatidae	1	0	0	0	1
Dolichopodiodidae	0	1	0	0	1
Gomphidae	0	0	1	0	1
Lymnessidae	0	1	0	0	1
Planorbiidae	0	0	0	1	1
Tipulidae	0	0	0	1	1
Coenagnonidae	1	0	0	1	2
Ephemeridae	0	2	0	0	2
Hydroptilidae	0	1	0	1	2
Psephenidae	1	1	1	0	3
Calamoceratidae	1	1	0	2	4
Helicopsychidae	0	0	2	2	4
Hyalellidae	0	0	1	3	4
Libellulidae	2	0	0	2	4
Perlidae	0	0	4	1	5
Chironomidae	4	0	2	0	6
Gyrinidae	5	0	1	0	6
Leptohyphidae	0	2	4	0	6

Dytiscidae	6	0	0	1	7
Planarbiidae	2	0	3	2	7
Leptophlebiidae	1	3	5	2	11
Baetidae	2	2	5	3	12
Gerridae	0	1	6	5	12
Naucoridae	0	2	6	5	13
Elmidae	1	2	8	4	15
Ptilodactylidae	0	10	1	6	17
Hydropsychidae	10	13	7	14	44
Total	37	42	57	56	192

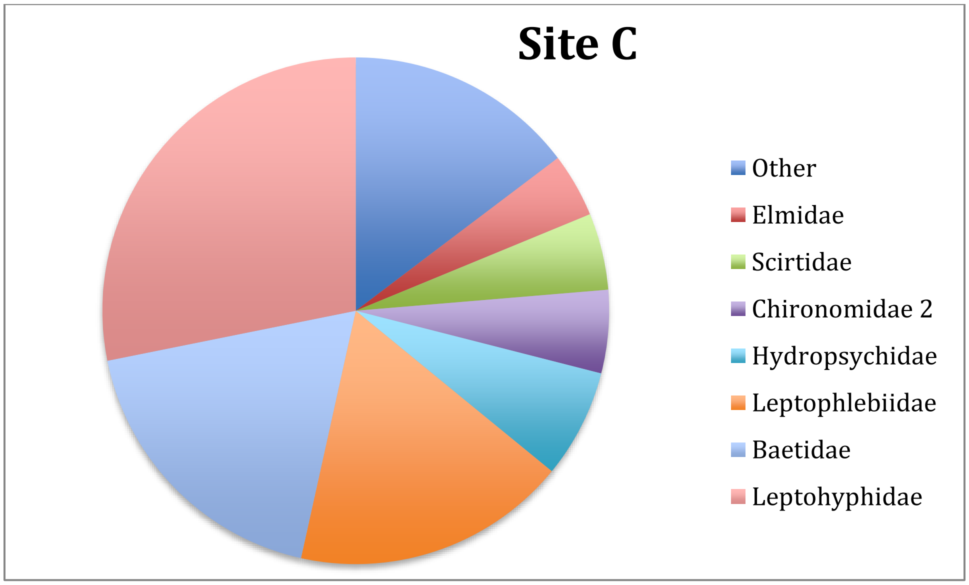


Supplemental Figure 3: The 7 most abundant species of macroinvertebrates at Site B, Lower Tupí, Mejía, Ecuador. N=192.

Supplemental Table 10: All of the macroinvertebrates found at Site C (Upper Pilatón River, Mejía, Ecuador), arranged from least abundant to most abundant. 2016.

C	11/8 + 11/9 C	11/12 C	11/15 C	11/16 C	11/22 C	11/23 C	Total
Assellidae	0	1	0	0	0	0	1
Belastomatidae	0	0	0	0	0	1	1
Ephemeridae	0	0	1	0	0	0	1
Gomphidae	0	0	0	0	0	1	1
Hydrobiosidae	0	0	0	0	1	0	1
Hydroptilidae	0	0	0	0	0	1	1
Libellulidae	0	1	0	0	0	0	1
Naucoridae	0	0	0	0	0	1	1

Notonectidae	0	0	0	0	0	1	1
planarbiidae	1	0	0	0	0	0	1
Psychodidae	0	0	0	0	1	0	1
Sialidae	0	0	0	0	1	0	1
Leptoceriade	0	0	0	1	0	1	2
Psephenidae	0	0	0	1	0	1	2
Perlidae	0	0	0	1	2	0	3
Caenidae	0	1	0	2	1	0	4
Simuliidae	0	3	1	1	0	0	5
Blephariceridae	0	0	0	7	1	0	8
Elmidae	0	0	0	2	7	1	10
Scirtidae	1	1	0	1	0	9	12
Chironomidae	2	2	0	0	3	6	13
Hydropsychidae	5	0	1	7	4	0	17
Leptophlebiidae	11	0	3	9	10	10	43
Baetidae	1	8	3	10	12	11	45
Leptohyphidae	6	6	3	6	15	33	69
Total	27	23	12	48	58	77	245

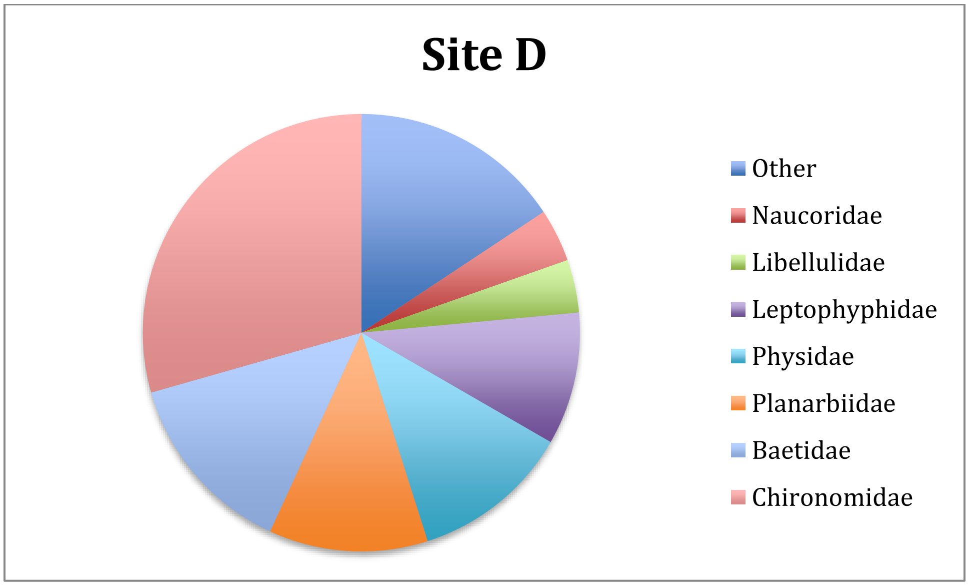


Supplemental Figure 4: The 7 most abundant species of macroinvertebrates at Site C, Upper Pilatón, Mejía, Ecuador. N=245.

Supplemental Table 11: All of the macroinvertebrates found at Site D (Intersection of Tupí and Pilatón Rivers), arranged from least abundant to most abundant. 2016.

D	11/13 D	11/18 D	11/19 D	11/20 D	Total
Naididae	1	0	0	0	1
Corydalidae	0	1	0	0	1
Stratiomyidae	0	1	0	0	1
Tipulidae	0	0	1	0	1
Elmidae	0	0	1	0	1
Calopterygidae	0	0	1	0	1
Leptophlebiidae	0	0	0	1	1
Hyalellidae	0	0	0	1	1

Naucoridae	1	0	0	1	2
Libellulidae	0	0	1	1	2
Leptophyphidae	0	2	1	2	5
Physidae	0	1	3	2	6
Planarbiidae	0	2	4	0	6
Baetidae	3	0	1	3	7
Chironomidae	3	3	3	6	15
Total	8	10	16	17	51

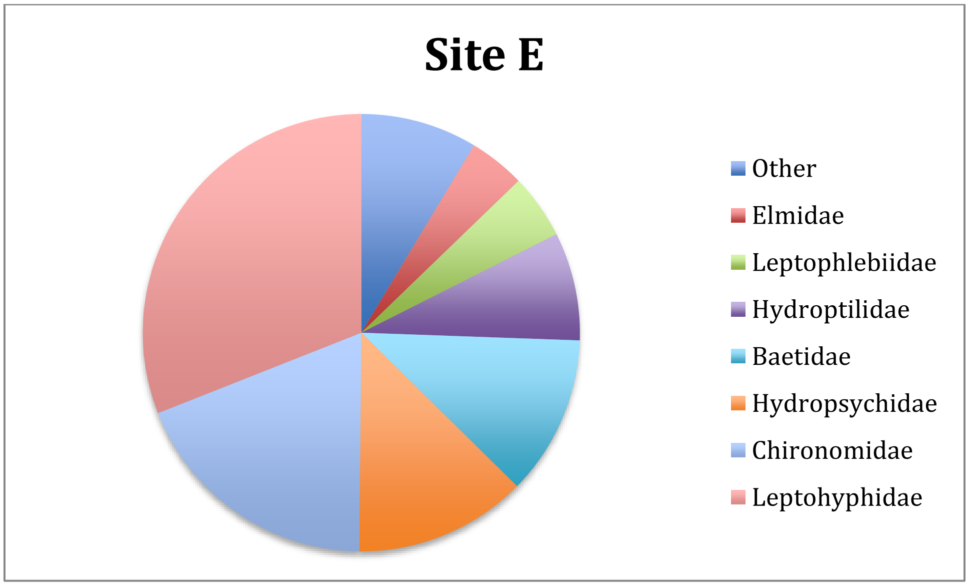


Supplemental Figure 5: The 7 most abundant species of macroinvertebrates at Site D, Intersection of Pilatón and Tupí Rivers, Mejía, Ecuador. N=51.

Supplemental Table 12: All of the macroinvertebrates found at Site E (Lower Pilatón River, Mejía, Ecuador), arranged from least abundant to most abundant. 2016.

E	11/24 E	11/26 E	11/27 E	11/27 E	Total
Dytiscidae	1	0	0	0	1
Corydalidae	0	1	0	0	1
Leptoceridae	0	1	0	0	1
Dolichopodiodae	0	0	1	0	1
Libellulidae	0	0	0	1	1
Helicopsychidae	0	2	0	0	2
Caenidae	1	0	1	1	3
Physidae	1	1	1	1	4
Hydrobiosidae	0	0	1	4	5
Philopotamidae	0	3	3	2	8
Elmidae	1	2	3	7	13
Leptophlebiidae	6	3	4	2	15

Hydroptilidae	4	2	9	10	25
Baetidae	16	11	9	1	37
Hydropsychidae	11	10	10	9	40
Chironomidae	20	9	11	19	59
Leptohyphidae	27	33	18	19	97
Total	88	78	71	76	313

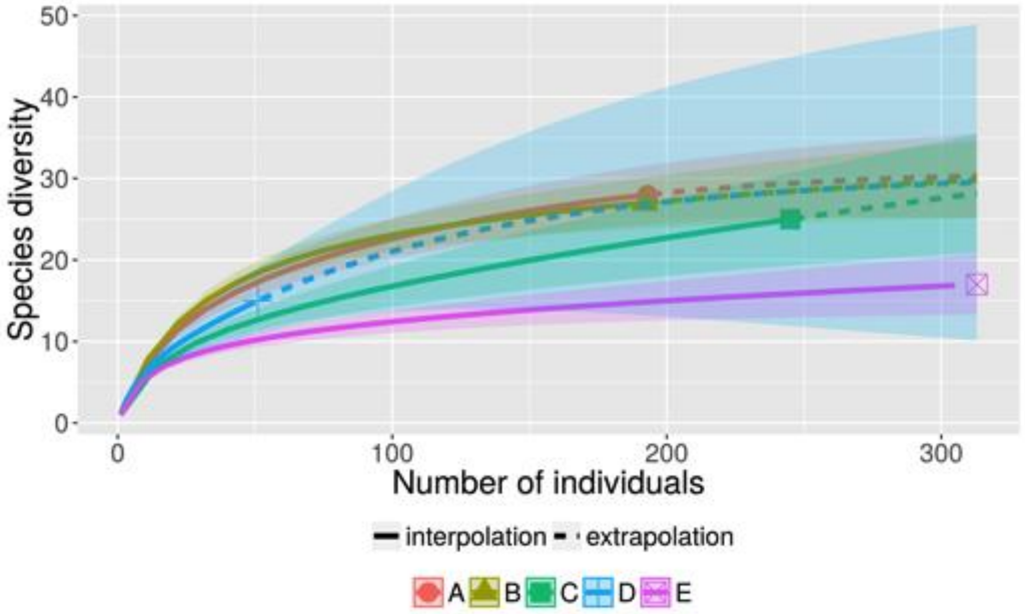


Supplemental Figure 6: The 7 most abundant species of macroinvertebrates at Site E, Lower Pilatón River, Mejía, Ecuador. N=313.

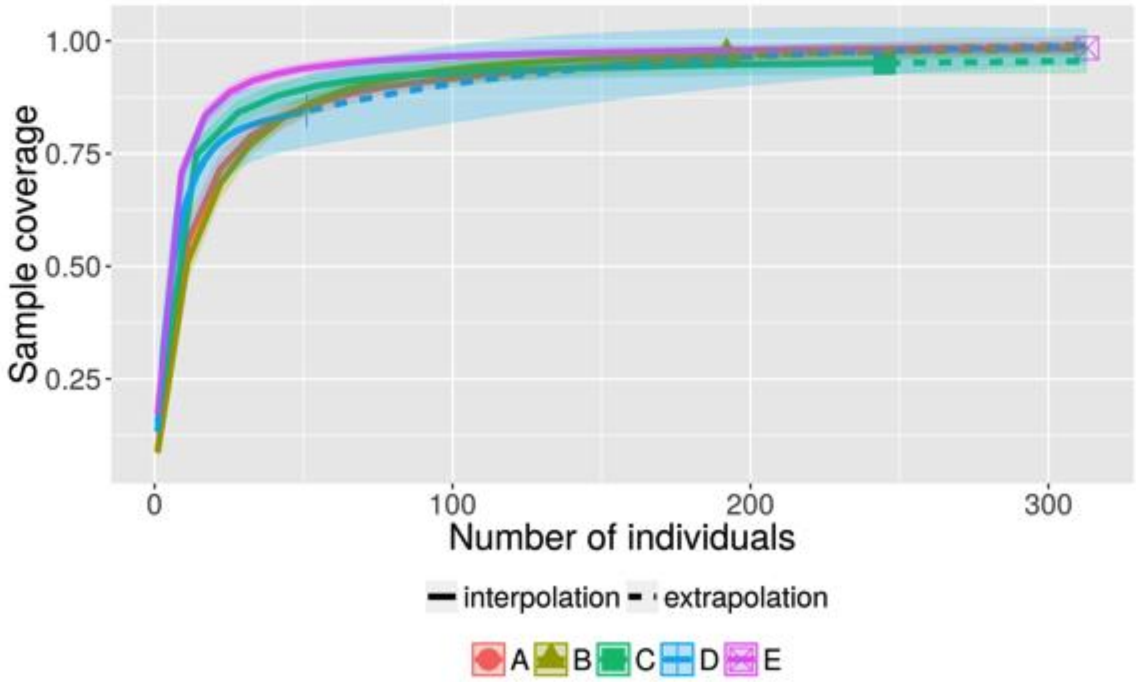
Appendix E: Diversity and Completeness

Supplemental Table 13: Number of samples collected (n), Families observed (S.obs), and estimated coverage of the data (C.hat) for each site using Chao, A., Ma, K. H., and Hsieh, T. C. (2016) iNEXT (iNterpolation and EXTrapolation) Online.

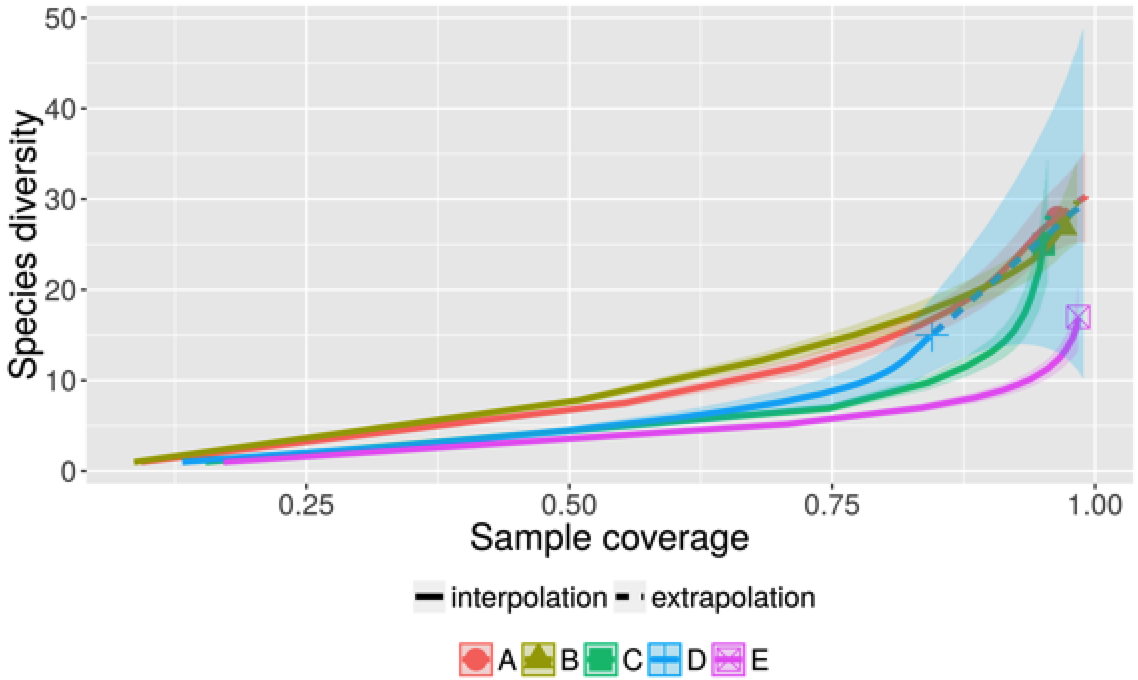
	A	B	C	D	E
n	193	192	245	51	313
S.obs	28	27	25	15	17
C.hat	0.9642	0.9689	0.9511	0.8447	0.984



Supplemental Figure 7: Sample-size-based rarefaction and extrapolation sampling curve for each site using Chao, A., Ma, K. H., and Hsieh, T. C. (2016) iNEXT (iNterpolation and EXTrapolation) Online.



Supplemental Figure 8: Sample completeness curve for all data from all sites using Chao, A., Ma, K. H., and Hsieh, T. C. (2016) iNEXT (iNterpolation and EXTrapolation) Online.



Supplemental Figure 9: Coverage-based rarefaction and extrapolation sampling curve using all macroinvertebrate families from each site, calculated with iNExt Online (Chao et al., 2016)

Appendix F: Images of Contamination

Supplemental Figure 10: Size and color of the Pilatón River at site C, Mejía, Ecuador.

Supplemental Figure 11: Gasoline Residue at site C on the Pilatón River, Mejía, Ecuador.

Supplemental Figure 12: Organic and inorganic waste by the side of the Tupí River, site D, Mejía, Ecuador.

Appendix G: Site observations log

Supplemental Table 14: Time, Weather, Width, Depth and Speed observations

Date/place	Time	Weather	Width (m)	Depth (m)	Speed
11/7 A	9:30-11:30am	Cloudy, rained last night	0.5-1.5	0.25-0.5	slow to moderate
11/8 C	9:10-9:40	Cloudy, poured last night	10	0.25-0.5	Moderate
11/8 B	10-10:30	Cloudy, poured last night	3	0.11-0.25	Slow to moderate
11/9 B	9:10-10:45	Cloudy, lots of rain last night	1-2	0.25-0.5	Moderate to fast
11/9 C	10:50-11:20	Cloudy, lots of rain last night	10	0.5-0.75	Fast
11/10 A	10:00-11:30	Cloudy, after many days of rain	0.5-1.5	0.10-0.75	Fast to moderate
11/12 C	6:00-7:30	Cloudy, river swollen, lots of rain	10	0.25-1.5	Fast
11/13 B	10:00-11:30	Cloudy, been raining a ton	3.4	0.14	Fast
11/14 A	10:00-11:30	Cloudy, less rain	<1.2	0.75-0.05	Moderate
11/15 B	10:00-11:30	Sunny	2	0.15-0.7	Moderate to fast
11/16 C	9:00-10:30	Sunny	7	0.14-1	Moderate to fast
11/17 A	10:00-11:30	Sunny	1-1.	0.1-0.5	Moderate to slow
11/18 D	9:30-11:00	Cloudy	2	0.1-0.5	Moderate
11/19 D	6:00-7:30	Cloudy	2m	0.1-0.5	Moderate
11/20 D	9:30-11:00	Sunny	2m	0.1-0.5	Moderate
11/21 B	9:30-11:00	Sunny	1-2m	0.1-0.7	Slow to moderate
11/22 B	8:30-10:00	Sunny	1-2m	0.1-0.7	Slow to moderate
11/22 C	10:00-11:30	Sunny	10m	0.2-1	Moderate to fast
11/23 C	9:00-10:30	Sunny	10m	0.2-1	Moderate to fast
11/24 E	9:00-10:30	Sunny	10m	0.2-1	Fast
11/25 A	9:00-10:30	sunny	.5-1m	0.2-1	Slow to moderate
11/26 E	6:00-7:30	Hazy	10m	0.2-1	Fast
11/27 E	6:00-11:00am	Hazy	10m	0.2-1	Fast

Supplemental Table 15: Substrate, cover, Bank, Condition (OM=Organic Matter) and color observations

Date/place	Substrate	Cover	Bank	Condition	Color
11/7 A	OM, rocks, leaves	Very covered	Natural	Dead leaves +OM	Transparent
11/8 C	Sand, rocks	None	Rocks	Some dead leaves	Transparent
11/8 B	OM, rocks	Very covered	Natural	Dead leaves	Transparent

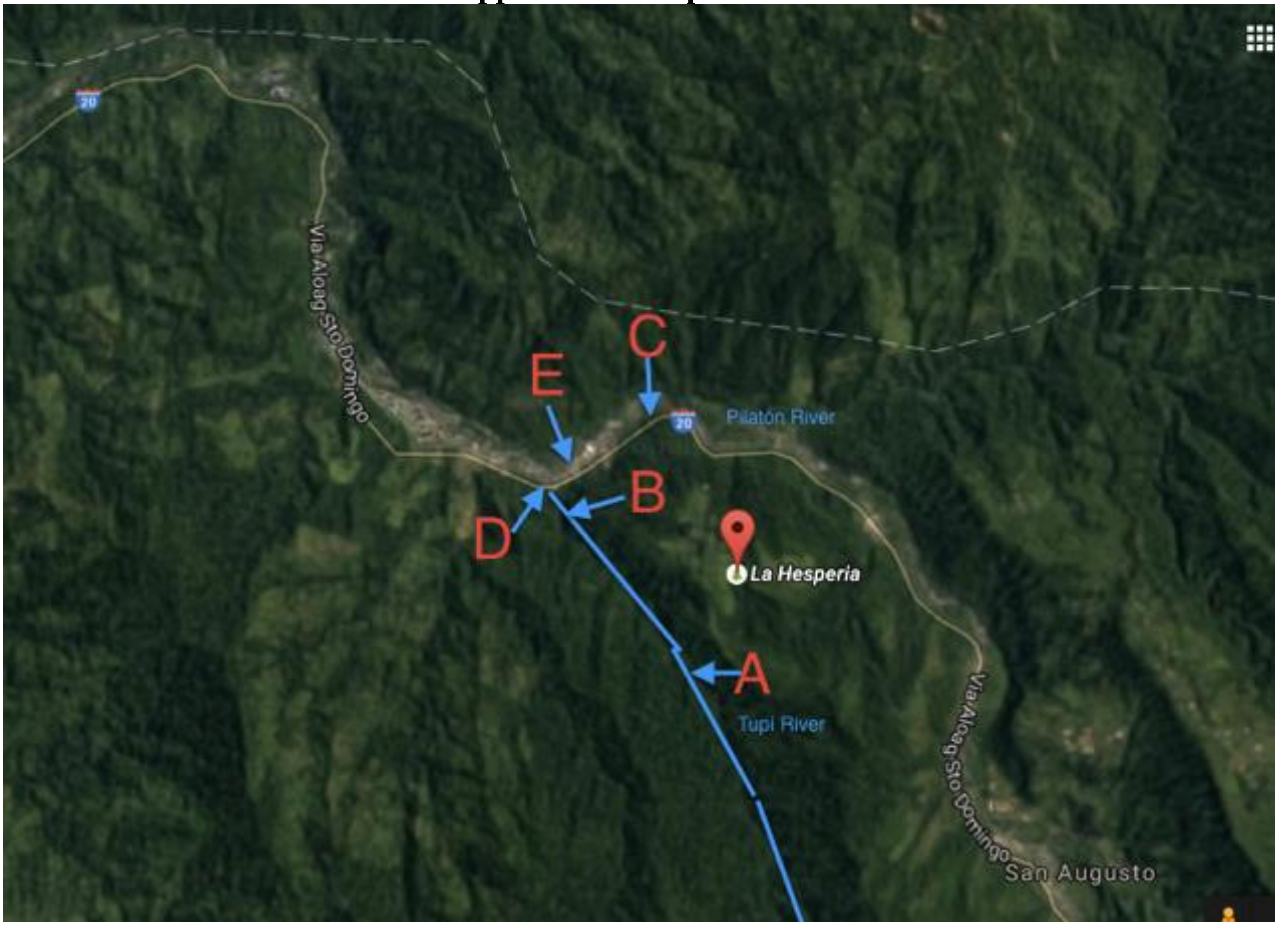
				+OM	
11/9 B	OM, rocks, leaves	Very covered	Natural	Dead leaves + OM	Transparent
11/9 C	Rocks, sand	None	Rocks	Some dead leaves	Brown
11/10 A	Rocks, sand, some dead leaves	Partially to very covered	Natural	Dead leaves, sand	Transparent
11/12 C	Rocks, some leaves	None	Rocks	Few dead leaves	Brown
11/13 B	Rocks	None	Natural	OM, dead leaves	Muddy brown
11/14 A	Rocks, OM, sand	Partially-very covered	Natural	OM + dead leaves	Transparent
11/15 B	Rocks, OM, sand	Very covered	Natural	OM + dead leaves	Transparent
11/16 C	Rocks and sand	None	Rocks	Dead leaves, mostly clean	Green/grey
11/17 A	Rocks, OM, sand	Partially covered	Natural	Dead leaves + OM	Transparent
11/18 D	Rocks and sand	None	Rocks, urban	OM, garbage	Brownish
11/19 D	Rocks, sand, OM	None	Rocks, urban	Lots of OM, garbage	Brownish
11/20 D	Rocks, sand, OM	None	Rocks, urban	Lots of OM, garbage	Brownish
11/21 B	Rocks, sand, OM, leaves	Very covered	Natural	Leaves + OM	Transparent
11/22 B	Rocks, sand, leaves, OM	Very covered	Natural	Leaves, OM	Transparent
11/22 C	Rocks, sand	None	Rocks, urban	Some dead leaves	A bit cloudy green
11/23 C	Rocks, sand	None	Rocks	Some dead leaves	A bit cloudy green
11/24 E	Rocks, sand, OM	None	Rocks, urban	OM	A bit cloudy green
11/25 A	Rocks, sand, OM, leaves	Very covered	Natural	Some OM, leaves	Clear
11/26 E	Rocks, sand, some OM	None	Rocks, urban	OM	A bit cloudy green
11/27 E	Rocks, sand, OM	None	Rocks, urban	OM	A bit cloudy green

Supplemental Table 16: Temperature (Air and Water), Sampling time, Aquatic Vegetation, Algae, Macroinvertebrate Abundance, and Surrounding Environment Observations

Date/place	Temp: Air	Water	Time	Aquatic vegetation	Algae	Macroinvertebrates	Surrounding
11/7 A	22	19.5	1.5 hrs	Some, rare	Some	Moderate	Forest
11/8 C	26	18	.5 hr.	Rare	Rare	Low	Gravel, concrete, some shrubs
11/8 B	24	19	.5 hr	Some	Some	Moderate	Forest
11/9 B	26	20	1.5	Some	Some	Moderate	Forest

			hr				
11/9 C	26	20	.5 hr	Rare	Rare	Moderate	Gravel, concrete, shrubs
11/10 A	22	18.5	1.5 hr	Rare	Some	Low	Forest
11/12 C	20	17.5	1.5 hr	Rare	Rare	Moderate/low	Gravel, concrete, some plants
11/13 B	19	17.5	1.5hr	None	Rare	Low	Urban
11/14 A	21	17	1.5 hr	Some	Some	Moderate	Forest
11/15 B	22	19.5	1.5 hr	Some	Some	Moderate	Forest
11/16 C	28	20	1.5hr	Rare	Some	Abundant	Gravel, rocks
11/17 A	26	20	1.5 hr	Rare	Some	Moderate	Forest
11/18 D	26	21	1.5hr	Some	Abundant	Rare	Urban
11/19 D	19	17	1.5hr	Some	Abundant	Rare	Urban
11/20 D	25	20	1.5 hr	Some	Abundant	Rare	Urban
11/21 B	25	18	1.5 hr	Some	Some	Moderate	Forest
11/22 B	20.5	18	1.5 hr	Some	Some	Moderate	Forest
11/22 C	23	17	1.5 hr	Rare	Rare	Abundant	Rocks
11/23 C	27	18	1.5 hr	Rare	Rare	Abundant	Rocks
11/24 E	25	18	1.5 hr	Some	Abundant	Moderate	Rocks
11/25 A	24	18	1.5 hr	Rare	Some	Moderate	Forest
11/26 E	23	17	1.5 hr	Some	Abundant	Abundant	Rocks
11/27 E	24	17	1.5 hr	Some	Abundant	Abundant	Rocks

Appendix H: Map



Supplemental Figure 13: Map of La Hesperia Natural Reserve, the Pilatón and Tupí Rivers, with sites A-E marked.